*Review Paper:*

# Biotechnological and physio-biochemical approaches in plant growth regulation by NPGRs –past and future perspectives

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## Abstract

*Growth and development of a plant is regulated by a diverse group of naturally occurring organic compounds known as Plant Growth Regulators (PGRs) or phytohormone having distinctive mechanism and regulatory properties. Phytohormones are signaling molecules which synthesis is very location specific within the plant part and active in extremely low concentrations. Recently in addition to five classical or traditional phytohormone, some Novel Plant Growth Regulators (NPGRs) directly or indirectly take part in plant growth regulation either at the cellular, molecular, morphological as well as physio-biochemical level. They affect gene expression and transcription levels, cellular division and growth of the plant.*

*At present, due to global warming issues as well as rapid increases in human population pressure, there is an insufficiency of food production. Therefore, an urgent need for enhancing agricultural production and productivity through a composite plant growth regulation based on biotechnological and physio-biochemical approaches in a sustainable manner and eco- friendly approach. Till today, a number of NPGRs are being used in plant cell, tissue and organ cultures. In this review, an attempted has made to summarize the remarkable progress based on the past and future perspectives towards understanding NPGRs in plant growth, development and metabolism at molecular, physio-biochemical level. The noteworthy improvement during the past towards the invention of the NPGRs (Novel Plant Growth Regulators) followed by their molecular mechanism of action in plant growth regulation are briefly reviewed herewith.*

**Keywords:** Novel Plant Growth Regulators (NPGRs), growth regulation and modulation, biotechnological, physio-biochemical approaches.

## Introduction

The five classical phytohormones are auxins, cytokinins, gibberellins, abscisic acid and ethylene that primarily involved in the plant growth regulation. They are small diffusible signaling molecules that easily penetrate between the plant cells. Interestingly, among all the classical naturally occurring PGRs, abscisic acid was the only regulator that chemically identified from plant tissue. Identification of most others came from the extracts of other plants. For an example, auxin was identified from urine and the fungal cultures of Rhizopus, gibberellins from culture filtrates of the fungus Gibberella, cytokinins from autoclaved herring sperm DNA and ethylene from illuminating gases[15].

However, recently in addition to these five traditional PGRs, several newer or novel compounds recognized as well as identified as classes of phytohormones that also known as Novel Plant Growth Regulators (NPGRs) such as brassinosteroids, jasmonic acid, salicylic acid signal peptides, morphactins, karrikins (KARs), strigolactone (SL), melatonin, serotonin, nitrobenzene, harzianolide, polyamines, traicontanol, oligosaccharides, sterols, systemins, the bioactive oligopeptides (such as CLE peptides) and various small proteins or peptides or peptide signaling molecules. These compounds have been discovered with biological activity which equals to or exceeds that of the equivalent endogenous PGR[24]. Further NPGRs mediated growth responses may lead towards the novel strategies of crop improvement.

**1. Karrikins (KARs):** Karrikins are a group of novel plant growth regulators (NPGR) identified as molecules derived from the smoke of burning plant material[11,19] and KARs have significant role in enhancing seed germination, plant branching etc. Karrikins are released into the air when plants are burning and deposited on the soil surface after rainfall. KARs is released in huge quantities as they are released from smoke[22]. KARs signaling mechanism and phenotypic responses has been well understood from the genomic structure of *Arabidopsis thaliana* L. First karrikin was discovered as $KAR_1$ that also known as karrikinolode[19].

**Chemical properties of Karrikins (KARs):** KARs is the first butenolide compounds that discovered in smoke water and its analogs were named as “karrikins” or KARs. The word “karik” used for smoke in Noongar Aboriginals’ language of South-west Australia[74]. The structure of KARs is very similar to another Novel PGRs (NPGRs), strigolactones (SLs). It can be quickly dissolved in organic solvents and also slightly in water[19]. KAR molecules are not synthesized from the plant but are produced by heating or combustion of carbohydrates like cellulose[21]. $KAR_1$, $KAR_2$, $KAR_3$, $KAR_4$, $KAR_5$ and $KAR_6$ are six KARs exhibited physiological activity in plants; almost 50 analogs of KAR1 with different substitutions have been synthesized[16,20,23]. Among the six KARs, $KAR_1$ to $KAR_4$ are the most active karrikins[41] and their structures are represented below:

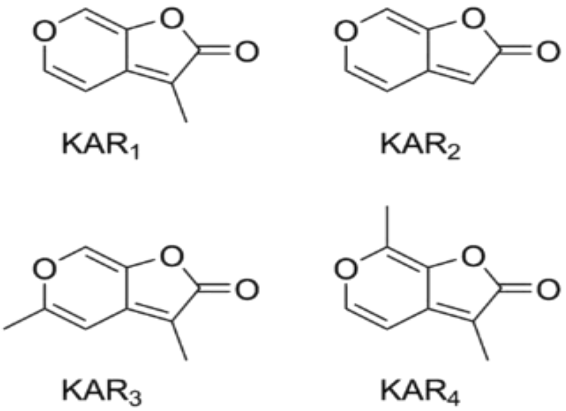


**Figure 1: Chemical structures of Karrikins (KARs).**
[Source: https://en.wikipedia.org/wiki/Karrikin].

**Molecular perception of KARs in plants:** Till date the exact mechanism of perception of KARs is not yet reported but genetic studies indicated that KARs is perceived by the KARRIKIN INSENSITIVE 2 (KAI2) receptor in *Arabidopsis*. KAI2 interacts with more auxiliary growth 2 (MAX2), which leads to complex degrading suppressor 2 (SMAX1) and SMAX1-LIKE2 (SMXL2). These reveal transcription factors from suppression and response to KARs[25,40,64,65].

**Function of KARs in plant growth regulation:** Karrikins not only stimulate seed germination, but are reported to increase seedling vigor[70]. They influence seedling photomorphogenesis which results in shorter hypocotyls and larger cotyledons reported in *Arabidopsis*. KAR treated photo-responses like inhibition of hypocotyl elongation and cotyledon expansion etc. that regulate germination. KARs involved in accumulation of chlorophyll a and b in *Arabidopsis thaliana* under continuous red light[39]. KAI2 protein is also required for leaf growth and development. KARs in combination with Strigolactones (SL) helps in the regulation of leaf morphology in *Arabidopsis*.

**2. Strigolactones:** Strigolactones (SL) are a new group of chemical compounds act as growth stimulant for weed species *Strigalutea* and it was first identified from plant's root[69]. They were first isolated from cotton plants, specifically from the cotton roots[13]. Later on they were distributed in *Striga* host such as sorghum, maize, proso millet etc.[23,37,60] *Orobranche* was also reported to use SL as germination stimulant[77]. Presently, two groups independently identified strigolactones well known as new branching-inhibiting hormones in rice, peas and *Arabidopsis*.

**Chemical properties of Strigolactones (SL):** Strigolactones comprise a diverse group with a common chemical structure[69]. They are highly soluble in polar solvents (acetone, benzene) and insoluble in hexane[13]. The structure of SL is based on a tricyclic lactone linked to a hydroxymethyl butenolide. Tricyclic lactone is represented as A, B and C whereas D represents the hydroxymethyl butenolide in figure 2[69].

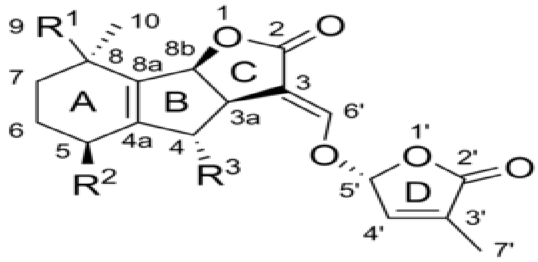


**Figure 2: General chemical structure of Strigolactones (SL).**
[Source: https://en.wikipedia.org/wiki/Strigolactone].

**Molecular perception of Strigolactones (SL) in plants:** Strigolactones are perceived by the dual receptor/hydrolase protein DWARF14 (D14), a member of the α/β hydrolase super family in plants. Hydrolases with poor substrate turnover is required for the protein's biological function[27].

**Function of SL in plant growth regulation:** Secondary plant growth is mainly regulated by IAA[59]. Strigolactones play a primary role in plant-fungi interaction[36] and plant stress responses including drought and salinity stress. SL lowers the ABA to GA balance and increases cytokinin that affects seed germination.

**3. Melatonin (N-acetyl-5-methoxytrypalmitine):** Melatonin (MEL) is a well-known animal hormone that first reported in plants during 1995. It has been detected and quantified in different plant parts (stems, shoots, laves, roots, fruits and seeds) in varying proportions[46,67]. Melatonin is rich in leaves of aromatic plants and in seeds but less in quantities. MEL is a pleiotropic molecule that performs important roles in plants as a growth regulator, circadian regulator as well as environmental stress protector. Remarkably, melatonin concentrations are high in coffee, tea, wine and beer and crops including corn, rice, wheat, barley and oats etc.[67]

**Chemical properties of Melatonin:** Melatonin (MEL) is an indole amine group of plant growth regulators (PGRs) that derived from tryptophan. MEL is a potent antioxidant and possesses resemblances with auxin as both have common precursor as tryptophan. It is biosynthesized from plants when they are exposed to both biological stresses, for example, fungal infection and non-biological stresses such as extremes of temperature, toxins, increased soil salinity, drought etc.[7,28,55]

**Molecular perception of Melatonin (MEL) in plants:** MEL decreased germination, root length and secondary root production, red light exposure increased root length and blue light decreased secondary root production. Such distinction has been supported by the recent discovery of a phytomelatonin 14 receptor PMTR1[75] as well as Hyp-1, a potential mediator of MEL action and MEL/cytokinin cross-talk which interacts with *NADPH Oxidases*, downstream of the PMTR1[62,63,76].

**Function of Melatonin in plant growth regulation:** Melatonin plays important role in reproductive development, circadian rhythm regulation, cell protection, wound healing and senescence, vegetative development as well as mitigating to both biotic and abiotic stresses etc.[6] It's have more scavenging capacity (antioxidant activity) against biological free radicals such as ROS and nitrogen species including the hydroxyl radical, singlet oxygen, peroxyl radical, hydrogen peroxide, peroxynitrite anion and nitric oxide etc.

Regarding its physiological function in plants, melatonin shows auxin activity and is an excellent antioxidant regulating the growth of roots, shoots and explants, enhances seed germination and plant productivity, chlorophyll preservation, promotion of phototsynthesis, respond to photoperiod and delaying leaf senescence. MEL has the ability to strengthen plants against abiotic stress (drought, cold, heat, salinity, chemical pollutants and herbicides etc.). UV radiation makes melatonin an interesting candidate for use as a natural bio-stimulating substance for treating field crops.

High melatonin content in transgenic plants is responsible to increase crop production. For an example, transgenic rice seedlings with elevated melatonin were found to be more resistant to herbicide induced oxidative stress than their wild type counter parts[48]. Increase in level of melatonin due to oxidative stress was reported in various plants[3,5,9]. They have protective role against UV and ozone damage[47,66,73].

**4. Serotonin:** Like melatonin, Serotonin (SER) [5-hydroxytryptamine (5-HT)] was also discovered as animal hormone. Serotonin is found in mushrooms, fruits and vegetables. It was first reported during 1954 in leguminous medicinal plant, cowhage (*Mucuna pruriens* L.) and later it has been identified in over 90 species and 37 plant families[57] but its concentration varies between plant families, species, cultivars, tissue as well as stage of maturity. SER has been recently recognized as a plant hormone due its auxin like activity and its biosynthesis also involves tryptophan. SER is found in varying quantities in different parts of plant species including leaves, stems, roots, fruits and seeds etc.

The highest values of 25–400 mg/kg have been found in walnut (*Juglans regia*) and hickory (*Carya spp*). Serotonin concentrations of 3–30 mg/kg have been found in pineapples, banana, kiwi fruit, plums and tomatoes. Moderate levels from 0.1–3 mg/kg have been found in a wide range of tested vegetables[18].

**Chemical properties of Serotonin:** Serotonin is an indole amine compound and potent antioxidant like MEL. Serotonin production in drying seeds is a way to get rid to buildup of poisonous ammonia. The ammonia is collected and placed in the indole part of L-tryptophan which is then decarboxylated by tryptophan decarboxylase to give tryptamine, which is then hydroxylated by a cytochrome P450 monooxygenase, yielding serotonin[58].

Biochemically, the indole amine molecule derives from the amino acid tryptophan, via hydroxylation of the 5 position on the ring (forming the intermediate 5-hydroxytryptophan) and then decarboxylation to produce serotonin[18].

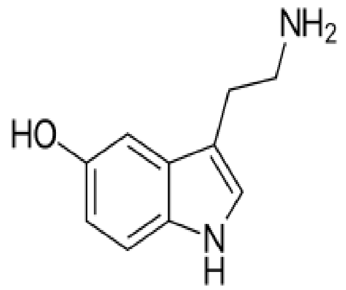


**Figure 3: General chemical structure of Serotinin (SER).**
[Source: https://en.wikipedia.org/wiki/Serotonin].

**Molecular perception of Serotonin (SER) in plants:** Both auxin and serotonin exhibits structural similarities which bring possibilities of auxin receptors being surrogated for serotonin transport in plants. Serotonin and auxin-responsive genes interactions have suggested their antagonistic nature.

Serotonin receptor-mediated signaling in plants is plausible till date but serotonin localized specifically to the vascular tissues of organs including somatic embryos, mature roots, stem and immature fruits have been reported in *Coffea canephora* Pierre ex A. Froehner[52].

**Function of Serotonin in plant growth regulation:** SER has been implicated in diverse physiological functions in plants viz. growth regulation[38], flowering, protective role as an antioxidant, xylem sap exudation[32], ion permeability[42], ripening regulation and plant morphogenesis.[14,52] SER exhibits the photomorphogenetic mediated response in plants and essential signaling molecule in diverse processes like protection of germ tissues and promotion of reproductive processes, mediation of plant rhythms including energy acquisition and senescence. For an example, SER stimulates phosophoinositide (PI) turnover, which is found to mimic the red light effect in enhancing the *nitrate reductase (NR)* transcript levels and inhibiting *phyI* transcript accumulation and releasing second messengers in maize[10,51]. SER plays an important role in photo-response in plants and thereby regulates circadian and seasonal rhythms.

SER also plays a protective role in plants due to their antioxidant activity by scavenging reactive oxygen species (ROS). SER plays a crucial role in delaying senescence, in maintaining the cellular integrity of xylem parenchyma and companion cells by protecting them from the oxidative damage and thus facilitate efficient nutrient recycling from senescing leaves into sink tissues[32].

They are also involved in vegetative growth and morphogenetic response such as promoting shoot production, growth and multiplication, biomass accumulation, delay in senescence, seed germination and somatic embryogenesis. It also stimulates the germination of both radish seeds[57] and the pollen of *Hippeastrum hybridum*[56]. It can detoxify excess ammonia and involves in many physiological functions including photosynthesis, reproduction and mediation and survival in response to biotic and abiotic challenge, reduces chlorophyll loss, membrane lipid peroxidation, increased reactive oxygen species (ROS) and induced senescence-related genes.

SER is believed to play a protective role against ROS leading to delay senescence as reported by analyzing *tryptophan decarboxylase* (TDC) over expression and TDC RNA interference (RNAi) in transgenic rice[31].

**5. Harzianolide:** Harzianolide is a metabolic component of bio-control agent isolated from *Trichoderma harzianum* that was studied for its role in plant growth and systemic resistance. They also exhibits auxin like activity and influences early seedling growth.

**Chemical properties of Harzianolide:** Harzianolide ($C_{13}H_{18}O_3$) chemically a 3- (2'- hydroxypropyl)-4-(hexa-2', 4'- dienyl)- 2(15H)- furanone by NMR methods[12]. It has also been isolated from three different strains of *Trichoderma harzianum*[1,12,45].

**Function of Harzianolide in plant growth regulation:** Harzianolide, the secondary novel regulator of *Trichoderma* which promotes plant growth through better root development and activation of plant defense mechanisms. They also enhance seedling growth in tomato, canola and wheat and show an auxin like activity on etiolated pea stems[71,72]. Antifungal activities against several plant pathogens have been reported and plant growth promotion effects were also detected for harzianolide[73].

**6. Nitrobenzene:** Nitrobenzene is a combination of nitrogen and plant growth regulators that extracted from sea weeds. It acts as plant energizer, flowering stimulant and low cost yield booster[8]. Nitrobenzene is quickly absorbed into the plants that influences the biochemical pathway of the plants to increases the nutrient use efficiency. Nitrobenzene improves the vegetative growth and induces profuse flowering and helps in the retention of the flowers and fruits[17]. Nitrobenzene 20% was used to repair the hormonal function of a plant which boosts the flowering and root growth[48].

**Chemical properties of Nitrobenzene:** It is the simplest aromatic nitro compound with chemical formula $C_6H_5NO_2$. This organic compound is water-insoluble but soluble in organic solvent (acetone, benzene, diethyl ether and ethanol). It is greenish yellow crystal or pale yellow oily liquid with an almond-like or bitter smell. It has melting point 5.7°C, boiling point 210.9°C and density 1.987 g/cm$^3$ at 25°C. It is produced on a large scale from benzene as a precursor to aniline. Biochemical effects of the nitrobenzene include detoxification of active oxygen, reduced gibberellin synthesis and increased chlorophyll content[61]. Nitrobenzene 20% v/v also used for increasing the number of fruits by sustaining the flower bloomed.

**Function of Nitrobenzene in plant growth regulation:** Nitrobenzene promotes flowering in plants and enhance production by preventing flower shedding. Nitrobenzene 20% v/v uses as flowering stimulant and also prevent flower shedding[17]. Fruit size and quality also be increase by enhancing early flowering as well as increasing the number of flowers. Maximum fruit weight was found by the foliar application of nitrobenzene on strawberry[43].

It has positive influence on taste, color, flavor and vitamin content of some vegetable crops which improves the quality and the commercial value of the products. Nitrobenzene is specially recommended for vegetable crops and flowering plants[33] as they have capacity to increase flowering in plant and also prevent flower shedding. In flowering plant, nitrobenzene enhances the color, appearance, size of a flower and enhancing plant cover which profound flowering thus increasing the quality and yield. It also increases the keeping quality of the produce of several crops.

It helps to increase the carbon and nitrogen ratio in plants and enhances the plant canopy as well as induces flowering. It can be used as foliar application (2-3ml/litre water) in all vegetables to increases their production and productivity and the most effective nitrobenzene concentration to reduce cost of production in order to improve the profit. It is compatible with pesticide and fungicides also. Nitrobenzene increases water holding capacity by stimulating root growth and reduces soil erosion. It also has some protective function against biotic and abiotic stresses.

Nitrobenzene can be used as spray in a suitable formulation (20% or 35%) or in granular form increases flower forming substances by altering auxin, cytokinin, gibbrellic acid and ethylene ratio favorably tilting to a higher level of flower forming substances, thereby increasing flowers by more than 40 to 45% and yield[54]. A higher nitrobenzene concentration along with Cytokinin and auxin also influences the lateral growth of parenchyma cells in stem so that the plant girth was increased[42,49].

Nitrobenzene application and their simultaneous transport to the auxiliary buds would have resulted in a better sink for the mobilization of photo-assimilates at a faster rate. This would have helped in the early transformation from the vegetative phase to reproductive phase[34]. It is used widely as a growth retardant in agricultural and horticultural crops, especially, on cereal crops, to prevent their lodging and decrease grain loss at ripening and develops plant tolerance to environmental stress, without affecting growth and productivity of the crop[35].

**Modulation of endogenous NPGRs level:** Phytohormone plays an important role in the growth and development of plants. Plants have the capacity to modulate their endogenous phytohormone levels to adapt themselves under any type of stress. During the entire life cycle of a plant, they can limit their growth and metabolism to adjust themselves when they are often subjected to a number of nonlethal stresses. Plant may also produce or modulate phytohormone and thereby alters phytohormone levels and thereby balance their hormonal levels in response to stress.

**Molecular and biotechnological approaches in growth regulation:** Biotechnological as well as molecular approaches like genetic engineering, soma-clonal variation to improve several desired traits in plants under *in-vitro* systems. Modern plant molecular biologists and biotechnologists have tried to improve outputs from plants and thereby regulate plant growth. The use of PGRs as a way of improving plant yield through micro propagation and somatic embryogenesis is the main focus for crop improvement. All these approaches could play a decisive role in agriculture because of its ability to directly modify plants, animals and agricultural processes in response to new needs[68]. They provide a method for the mass clonal propagation of plants via regeneration under *in-vitro* condition. Plant tissue culture techniques are also a tool for their germplasm conservation as well as for reforestation and tree improvement[2,50].

Micro-propagation is an alternative to other propagation methods lies in its ability to multiply elite clonal materials very rapidly. It has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell culture, tissue and organ culture on well-defined tissue culture media in presence of plant growth regulators under aseptic conditions[30]. The genetic potential of a cell to regenerate a whole plant has been termed as 'totipotency' and perhaps was the driving force which led German physiologist G. Haberlandt to conceive the idea of plant tissue culture that leads to the use of NPGRs.

## Conclusion

In the more developed world, where PGRs as well as NPGRs and other agricultural chemicals are relatively inexpensive, the use of chemicals occupies a better regulation in plant growth and development. In the recent future, a great understanding of these chemicals in regards to molecular as well as physiobiochemical mechanisms of growth regulator biosynthesis, perception and response plays a significant role in stimulation of plant growth and regulation.

However, a balance between the modes of action of these NPGRs with classical phytohormone is adjusted to produce a normal plant by controlling its transport, distribution and signal transduction. These NPGRs are commonly used as biotechnological approaches in a number of different techniques involving plant propagation from cuttings, grafting, micro propagation and tissue culture etc. All of these approaches lead to develop stress tolerant varieties having high productivity and better adaptability towards the changing climate.

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